Comparative efficacy of antibiotic sensitivity tests for management of acute clinical *Escherichia Coli* mastitis in crossbred cow

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Abstract

A seven year old Holstein Friesian crossbred cow parturited two and half month ago in her fourth lactation was presented at the clinics with severe enlargement of udder. The mastitis was diagnosed on the basis of swollen, redden and painful udder with change in quality and production of milk as well as electrical resistance measuring through DRAMINSKI Mastitis Detector. The milk sample was collected aseptically from severely affected left hind quarters and antibiotic sensitivity test using commercially available kit (MastiTest™) was performed wherein Gentamicin and Ciprofloxacin were highly sensitive whereas sensitivity of Enrofloxacin was intermediate after 24 hours of inoculation at room temperature. *Escherichia Coli* was isolated on the basis of gram negative cocco-bacilli on microscopic examination and lactose fermentative on MacConkey agar as well as Metallic seen on Eosin Methylene Blue agar. The antibiotic sensitivity by disc diffusion method using Mueller-Hinton agar the basis of zone of inhibition revealed the highest sensitivity of Gentamicin followed by Ceftriaxone and Enrofloxacin. The cow was treated with intra-mammary and systemic injections of Gentamicin for 5 consecutive days and showed recovery within few days except left hind quarter among affected quarter in both milk quality and production.

Keywords: Antibiotic sensitivity; *Escherichia Coli;* Mastitis; Cow

Introduction

Mastitis is the most common and economically important disease of dairy cow and buffaloes in our country. It causes losses because of lower milk production but also by discarded milk, loss of stimulation, treatment cost and its effects on reproduction. A variety of bacteria, fungi, yeast can be isolated from bovine mastitis cases. However, *Staphylococcus aureus* and *Escherichia coli* are the most common causes of contagious and environmental clinical mastitis, respectively (Chandrasekaran *et al*., 2014). Isolation and determination of causative pathogens is still a gold standard (Radostits *et al*., 2000), but partly due to geographical distances between farms and laboratories, milk samples are often not sent to the laboratories as well as most of cases are treated by veterinarians according to their field experience. The antibiotics are routinely practiced as therapeutic measures of this malady but indiscreet use of antibiotics develops resistance in animal body which lowers cure rate (Barkema *et al*., 2006). That all can lead to low therapy success and increased risk to human health due to uncontrolled use of antibiotics. Beside, available methods of antibiotics sensitivity are cumbersome in field conditions. Therefore, the comparative efficacy of the commercially available antibiotic susceptibility test kit (MastiTest™,Himedia, Mumbai, India) and *in-vitro* disc diffusion methods were studied.

Materials and Methods

History and Clinical observations

A seven year old H. F. crossbred cattle, parturated normally two and half months ago was brought with complaint of sudden drop in milk production and swelling of left hind quarter of udder; treated locally by antibiotic (Inj. Wocef- Tz @ 4gm IM) and supportive therapy but without improvement, progressive infection affecting the other quarters except right hind. Cow was depressed with slight rise of rectal temperature (103 °F). Clinically, udder was swollen, reddish and painful to touch with watery, viscous yellow milk. DRAMINSKI Mastitis Detector (*DRAMIŃSKI* Ul. Owocowa 17, 10-860 Olsztyn, Poland) was used to measures changes in the electrical resistance of milk, as development of sub-clinical mastitis (asymptomatic stage) is accompanied by an increase in salt levels in milk, which involves a change in the resistance. It revealed the left hind (230) infected severely followed by left fore (250) and right fore (300), whereas right hind (380) quarter was normal. Milk sample was collected aseptically from left hind quarter to perform antibiotic susceptibility test (MastiTest™), isolation of bacteria and antibiotic sensitivity by disc diffusion method.

Organism isolation

Primary bacterial isolation was carried out by standard microbiology technique, milk sample was introduce on brilliant green agar and incubated at 37° C for 24 hours. Isolated colonies were stained with Grams method and observed gram negative, cocco-bacilli under light microscope at 10 X. Further, primary isolation of coliform bacteria was done on macConkey agar (MCA) and eosin methylene blue (EMB) agar as a differential medium and incubated aerobically at 37°C overnight. Pink colonies on MCA were positive lactose fermentation. Further the Lactose fermenting colonies were sub cultured on EMB agar medium and found green metallic sheen indicative of *Escherichia Coli* bacteria.

Antibiotic sensitivity tests

MastiTest™ antibiotic sensitivity test kit

The principle of MastiTest™ kit (Himedia Laboratories Pvt. Ltd., A-406, Bhaveshwar Plaza, Mumbai – 400 086 , India) is based on inoculation of milk sample into the control vial and antibiotic vials, and observing the visual colour changes from the original blue colour due to the presence of positive organism. It was performed on first day according to manufactures specification: 5 ml of milk sample was inoculated into sample vial, mixed properly and 9 ml (test sample) was drawn into sterilized syringe. Then, 1 ml of test sample was inoculated into control vial, likewise 1 ml of test sample was transferred into test vials namely T1, T2, T3, T4, T5, T6, T7 and T8. Once the test vials and control vial were injected with the teat sample, the vials were shaken to reconstitute the content of vials (Figure 1). The antibiotic was preferred on the basis of minimum loss of original blue colour to colourless/ white/ yellow, the Gentamicin (T3) and Ciprofloxacin (T5) were highly sensitive and Enrofloxacin (T4) was intermediate whereas all others were resistant after 24 hours of inoculation at room temperature (Figure 2).

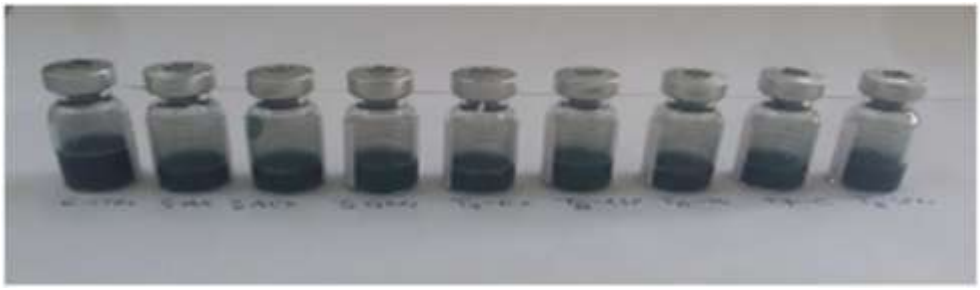

Fig 1. MastiTest™ immediately after inoculation of milk sample
Where, C- CTRL: Control, T1- AX: Ampicillin/Cloxacillin AX 128/128 mcg, T2 – ACX: Amoxycillin/Cloxacillin ACX 128/128 mcg, T3 – GEN: Gentamicin GEN 128 mcg, T4 – EX: Enrofloxacin Ex 8 mcg, T5 – CIP: Ciprofloxacin CIP 8 mcg, T6 – TE: Tetracycline TE 128 mcg, T7 – C: Choramphenicol C 8 mcg, T8 – SPN: Streptomycin/Penicillin SPN 128/128 mcg

Figure 2. MastiTest™ after 24 hours of inoculation of milk sample

In-vitro disc diffusion Antibiotic sensitivity test

Antibiotic sensitivity survey of isolate was also investigated by disk diffusion method in which the *E. Coli* colony were picked and seeded with standard amount of organism on Mueller–Hinton medium. Seven different commonly used antibiotics in veterinary practice, antibiotic discs (Himedia, Mumbai) were placed on the surface of that medium for their sensitivity and incubated at 37 ºC for 24 hours. Subsequently, the plates were examined for the development of zone of inhibition around the discs and interpreted. Wherein, *E. coli* showed more sensitivity to Gentamicin (GN– 10 mcg) followed by Ceftriaxone (CT– 30 mcg) and Enrofloxacin (Ex– 10 mcg), whereas resistant to Ciprofloxacin (CP– 30 mcg), Tetracycline (T– 30 mcg), Chloramphenicol (CL– 25 mcg) and Penicillin (P– 10 units) antibiotic discs (Figure 3).

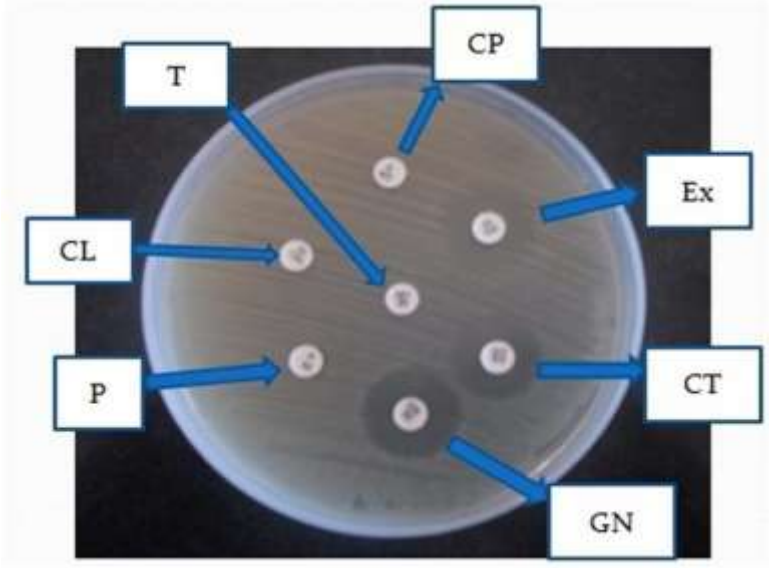

Figure 3. Zone of inhibition in In-vitro disc diffusion

Results and Discussions

According to Petrujkić et al.,(2010) and Rodostits *et al*., (2000), various classical methods for detection of mastitis includes viz. determination of somatic cell count, Nagase test (inflammation markers of milk i.e. enzymes N-acetyl-β- D-glucosaminidase and lactate dehidrogenase), measuring electric resistance of milk, california mastitis test, hymast diagnostic kit, ELISA and finally isolation of causative agent and antibiograme. Out of them, electrical resistant and California mastitis test are considered to be the most reliable indirect test in diagnosing subclinical mastitis (Rodostits *et al*., 2000). The DRAMINSKI Mastitis Detector measures the changes in the electrical resistance of milk, as development of sub-clinical (asymptomatic stage) or clinical mastitis were accompanied by an increased in salt levels in milk, which involves a change in the resistance.

Bovine mastitis is caused by various species of gram positive and negative micro-organism and includes contagious pathogens, environmental pathogens, minor and uncommon pathogens which can be treated by using specific and selective antibiotics (Rodostits *et al*., 2000). In present study, *Escherichia coli* were isolated and identified as gram negative cocco-bacilli by gram stain, lactose fermenters on MCA and metallic sheen on EMB agar. The crossbred cow was treated with intra-mammary (10 ml) and systemic injections of Gentamicin (Inj. Vetamicin @ 4mg/kg body weight, Geevet Remedies, Mehsana) with supportive therapies for 5 consecutive days. Animal recovered within 15 days as the affected quarter except left hind, returned to normal apart from quality and production of milk.

The comparative efficacies of antibiotic sensitivity test for milk sample of commonly used antibiotics in field were depicted in Table. The concentration of the antibiotics was various in antibiotic disc and MastiTest ^{TM}kit, but result of the both tests were well comparable except the ciprofloxacin. The cow was locally treated with injection containing antibiotic Ceftriaxone without improvement but it showed the sensitive in disc diffusion method. It might be due to indiscriminate use of antibiotics which fails to reach minimum inhibitory concentration in blood.

Table. Comparative efficacies of antibiotic sensitivity tests

Antibiotics Disc	**Antibiotic concentration**		**Antibiotic sensitivity**	
	MastiTest^{TM}	**Disk diffusion**	**MastiTest^{TM}**	**Disk diffusion**
Gentamicin	128 mcg	10 mcg	Sensitive	Sensitive
Ciprofloxacin	8 mcg	30 mcg	Sensitive	Resistant
Tetracycline	128 mcg	30 mcg	Resistant	Resistant
Chloramphenicol	8 mcg	25 mcg	Resistant	Resistant
Enrofloxacin	8 mcg	10 mcg	Intermediate	Intermediate
Ampicillin + Cloxacillin	128/128 mcg	_	Resistant	_
Amoxicillin + Cloxacillin	128/128 mcg	_	Resistant	_
Streptomycin + Penicillin	128/128 mcg	_	Resistant	_
Penicillin	_	10 units	_	Resistant
Ceftriaxone	_	30 mcg	_	Sensitive

Gram negative pathogens were reported to be more sensitive to enrofloxacin and gentamicin and less sensitive to ampicillin and penicillin (Karthikeyan, 2003) which supported the present findings in both the antibiotic sensitivity tests. Ranjan et al., (2010) found that the *E. Coli* bacteria were highly to moderate sensitive to enrofloxacin, ciprofloxacin, chloremphenicol followed by ceftriazone and gentamicin antibiotics whereas amoxicillin, penicillin G, streptomycin were mild sensitive to resistant through disc diffusion method. According to Chandrasekaran et al., (2014), *E. Coli* showed more sensitivity to enrofloxacin (79%) followed by amoxicillin and sulbactam (74%), gentamicin (73.1%) and ceftriaxone (69%) and resistant to penicillin G (63%), oxytetracycline (47.95) and amoxicillin (52.1%). Petrujkic et al., (2010) used the MASTiK® AST kit (ImmuCell Corp., Portland, ME, USA) and Kirby-Bauer disc diffusion test and found that gentamicin, cefriazone and oxytetracycline were sensitive, ampicillin and streptomycin were intermediate and penicillin and amoxicillin were resistant to *E. Coli* bacteria by Kirby-Bauer disc diffusion test whereas oxytetracycline and ampicillin antibiotic sensitive and penicillin was resistant by MASTiK® AST kit.

The antibiotic usage has directly contributed to an increased prevalence of resistant *E. Coli* mastitis (Danmap, 2001). All antimicrobial use in the herd may affect the resistance of *E. coli* isolates by increasing the presence of these antimicrobial agents in the cow's environment. Indiscriminate use of antibiotic leads to resistance of mastitis causing bacteria. Hence, selection of antibiotic is necessary to advocate effective treatment.The incidence of resistant mastitis was higher which might be due to indiscriminate use of antibiotics and intramammary preparations containing combinations or alone broad-spectrum antibiotics (Pitkala *et al*.,

2007). Edward *et al*., (2002) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. It is therefore, very important to implement a systemic application of an antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections. Further, it highlights the need for preventing the indiscriminate use of antibiotics.

The result of study envisaged that commercial available antibiotic sensitivity test kit is simple and reliable for use in field conditions as its accuracy was comparable to the result obtained by disc diffusion method in selection of antibiotic for treatment of *E. Coli* mastitis in cattle. This approach can lower irrational use of antimicrobial drugs and lower risk of antibiotic resistance for animal and human population.

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